Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689233
USEPA DP Barcode: 435146
PMRA Data Code: 9.2.4.6
PMRA Study No. (UKID): 2627507
Data Requirement (Guideline): OECD Guidance Doc. No. 75

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.8%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

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Date: 15 February 2018

PMRA Reviewer: Vedad Izadi
Evaluation Officer, PMRA/EAD/ERSII
Date: 4 October 2017

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CITATION: Franke M. 2015. Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development. BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany. Report No. 421109. Sponsor: BASF SE. Report No. BASF Reg. Doc. #: 2015/1000402. USEPA MRID 496892-33. PMRA UKID 2627507.

Executive Summary:
The semi-field (tunnel) study tested the effects of the formulated end-use product BAS 440 00 I (9.8% afidopyropen) on honeybee (*Apis mellifera*) colonies with the intent of examining brood (*i.e.*, eggs, larvae, pupae) strength and colony strength (number and condition of adult bees/brood and available food reserves). The study design was based in part on OECD Guidance Document No. 75. Nucleus bee

colonies (containing 9802 ± 239[1] adult bees/colony) within individual enclosures containing phacelia (*Phacelia tanacetifolia*) in full bloom were exposed, while bees were both actively foraging (*i.e.* daytime application [afidopyropen I]) and while bees were not actively foraging (*i.e.* evening application [afidopyropen II]), to either 0.10 L/ha (10 g a.i./ha; 0.009 lbs a.i./A) of BAS 440 00 I, the insect growth regulator fenoxycarb (300 g a.i./ha), the organophosphate insecticide dimethoate (480 g a.i./ha), or a water (negative) control treatment. Each treatment group consisted of four replicate tunnels, each tunnel containing a single nucleus colony; colonies were acclimated to the tunnels four days before applications. Colonies were maintained in the tunnels for 7 days after treatments (DAT, "exposure phase"), and then transferred to a remote monitoring site without a bee-attractive flowering crop for 86 days ("monitoring phase"). Adult and larval/pupal mortality were recorded from four days before, to 93 days after, treatments (-4 to 93 DAT); assessments included foraging activity (-4 to 7 DAT), colony condition (food stores, brood status), colony strength (numbers of adults and pupae), and brood development indices (brood index, brood compensation index, and brood termination index) at 4, 8, 14, 19, 26, 69 and 93 DAT.

The preliminary brood check indicated healthy colonies with all brood stages present, and a sufficient supply with nectar and pollen. Throughout the study, the number of food or brood cells did not differ statistically among the three treatment groups. Treatment rates were not confirmed analytically and are therefore based on nominal treatment levels.

There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen (daytime or evening applications) treatment groups and the negative control during the pre-application or exposure phases of the study; during the monitoring phase, mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, lower by 15%) in daytime application afidopyropen tunnels compared to control tunnels. There was reportedly no mortality of pupae measured in afidopyropen-treated tunnels at any point in the study. There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen-treated (daytime or evening applications) tunnels and the negative control during the pre-application phase of the study, but during the exposure phase of the study, mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 27% lower) in daytime application afidopyropen tunnels relative to control tunnels. With the exception of one instance (19 DAT), there were no significant ($p > 0.50$) differences in colony strength (mean number of adult worker bees or pupae/colony/d) or condition (mean number of brood or food cells/colony/d) in test item (daytime or evening applications) tunnels relative to the negative control.

The mean brood index and brood compensation index were significantly ($p < 0.05$) different (*i.e.*, lower by 35-38 and 29-44%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies, and the mean brood termination rate was significantly ($p < 0.05$) different (*i.e.*, higher by 130-169%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies. Overall effects from evening applications of afidopyropen were similar to effects from daytime applications, though of slightly lower magnitude (*i.e.*, lower brood index and brood compensation index, and higher brood termination rate) but these effects were not significantly different from those in control colonies due to higher variance around treatment means. Finally, afidopyropen treatments resulted in sublethal behavioral effects after application on the day of treatment (0aa DAT) in the daytime test item application tunnels. Within 30 minutes of applications 10-

[1] Note that all means in this summary are followed by ± one standard error (SE).

30 bees in each tunnel were motionless, showed reduced ability to respond to stimulation, fell off of treated plants, exhibited impaired locomotion and cramping; these sublethal effects were reported to have occurred only through the end of the day of applications (*i.e*., 0 DAT).

Results Synopsis:
The study is generally consistent with OECD Guidance Document No. 75, although there are some potentially important study deviations and deficiencies. Treatment levels were not analytically verified in the study, and due to possible effects of weather prior to and immediately following applications, there is some uncertainty regarding actual afidopyropen exposure levels. However, magnitude of residue studies provide some evidence that bees were appropriately exposed to the test item treatments, and colonies were responsive to reference toxicant treatments, indicating that overall the study was conducted properly.

Honey bee colonies treated with formulated afidopyropen at 10 g a.i./ha (0.009 lbs a.i./A) during active bee flight exhibited significant ($p<0.05$) adverse effects on foraging activity, and brood development resulting in a no-observed adverse effect level (NOAEL) of <10 g a.i./ha under the conditions tested. Adverse effects on foraging activity occurred during the exposure phase of the study, and brood development was adversely affected throughout the study, suggesting that under the conditions tested there were prolonged treatment effects on honeybee colonies due to daytime afidopyropen applications. Afidopyropen applications during the evening when bees were not actively foraging had relatively minimal adverse effects on honeybee colonies; however, effects on brood development from evening applications of afidopyropen were similar to effects from daytime applications, though of slightly lower magnitude (*i.e.*, lower brood index and brood compensation index, and higher brood termination rate), but these effects were not significantly different from those in negative control colonies.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

I. DATA SOURCE

USEPA MRID No.:	49689233
PMRA UKID No.:	2627507
Study Title:	Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development.
Study Author(s):	Franke M.
Testing Laboratory:	BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany.
Laboratory Report No.:	421109
Sponsor Study No.:	BASF Reg. Doc. #: 2015/1000402
Study Completion Date:	17 December 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes

II. MATERIALS AND METHODS

Test Guideline:	OECD Guidance Doc. No. 75 (2007)

Deviations from Guideline:

- The quantities of material applied in both the test item (afidopyropen) and the reference items (fenoxycarb and dimethoate) treatments were not verified analytically.
- The acclimation period for honey bee colonies in this study (4 days) is longer than what is recommended (2-3 days) in OECD Guidance Document No. 75; though not explicitly stated by the study author, weather data indicates that it was relatively cool and cloudy for the several days before applications were made, which could explain the extended acclimation period (see Reviewer's Comments for additional discussion).
- On -2 and -1 DAT the mean daily temperature was 13.9-14.1 °C (minimum daily temperatures were 11.4-21.1 °C); additionally, cloud cover on these days was 100%. OECD Guidance Document No. 75 notes that daytime temperatures below 15 °C may inhibit honeybee foraging activity.
- Sublethal behavioral effects were apparently only observed and recorded for the two afidopyropen treatments, and only for 0-7 DAT. The absence of behavioral effects data for the negative control groups and the reference treatment groups means that it is not possible to identify whether sublethal effects reported for afidopyropen treatment group are actually treatment-related, or rather are due to some site-level conditions that might equally affect all treatments.

GLP Compliance: Yes; signed GLP certificate was included and reported no guideline deviations. Laboratory certified by the Staatsministerium fur Umwelt und Landwirtschaft, Freistaat Sachsen.

A. MATERIALS

Test Material: BAS 440 00 I (VERSYS™)

Test Material Identity Batch No. FD-130925-0022; a yellow, liquid formulation comprising afidopyropen (BAS 440 I): 100 g/L (nominal), 98.2 g/L (9.8% measured).

Details on Preparation and Application of Test Materials:
All substances were applied in 400 L/ha water using a calibrated, portable plot sprayer. Applications were made during the day to correspond with active bee flight (*i.e.*, test item I), and during the evening to avoid bee flight (*i.e.*, test item II); all applications were made to fully flowering phacelia (BBCH 63-65).

Analytical Monitoring: None reported.

Details on Analytical Monitoring:
N/A

Reference material I: Insegar™ (fenoxycarb: 250 g/kg [nominal]); batch no: SM01A404; grey solid (water dispersible granules)

Reference material II: BAS 152 11 I (dimethoate: 400 g/L [nominal]); batch no: FRE-000926; blue liquid (emulsifiable concentration)

Vehicle: None

Test Organism (Species): *Apis mellifera* L. (honeybee)

Animal Group: Arthropoda/Insecta/Hymenoptera/Apidae

Details on Test Organisms: Healthy honeybee colonies, containing eleven combs consisting of seven to ten brood combs including all brood stages and sufficient food

supply, were used for the study. At the first brood assessment, *i.e.*, brood fixation day zero (BFD 0) two days prior to treatment (-2 DAT), colonies contained 8,663-11,363 adult bees. Bees in the colonies were free of clear visual signs of disease or pests, and no unusual occurrences were reported in colonies prior to treatments. Sister queens from 2013 were used to produce colonies which were as uniform as possible (source: Bienenfarm Kern Rehbacher Anger 10, 04249 Leipzig-Rehbach, Germany).

B. STUDY DESIGN AND METHODS

Study Type: Semi-field (tunnel) study

Test Duration Type: Long-term toxicity test; duration of core study was 28 days, some additional assessments were conducted 69 and 93 DAT.

Limit Test: None reported

Total Exposure Duration: 7 d

Post-Exposure Observation Phase:
20 d for all endpoints except colony and brood strength (84 d)

Remarks: Bee mortality was assessed daily beginning two days before (-2 DAT) and ending at 27 DAT. Mortality in the tunnels was evaluated using linen sheets (area approximately 18 m^2) laid at ground level inside the front, middle and back of the tunnels, as well with dead zone dead bee traps at each hive entrance; mortality at the monitoring site was evaluated using only dead zone dead bee traps. Foraging activity of the bees, and overall behavior, were assessed -2 to 7 DAT. Overall condition of the colonies (food stores, brood status and colony strength) were assessed –2, 4, 8, 14, 19, 26, 69 and 93 DAT, while detailed brood assessments were made on -2, 4, 8, 14 and 19 DAT. Colony strength and condition assessments were conducted according to the assumption that the maximum number of bees per colony consisting of one super with a total of 11 combs and two bounding hive walls could theoretically be 21,600 bees. For assessments it was further assumed that each comb side was separated into 8 equal sections covered by a theoretical maximum number of 900 bees, assessments were conducted by counting the number of “eights” covered by bees (assuming that each eight held 112.5 bees), and then extrapolating the number of “eights” per comb to the estimated total number of bees per colony.

Detailed brood development of single brood cells was performed using the NEXTREAT digital image analysis tool, with brood frames (300 cells) containing eggs observed over one complete brood cycle of 21 days. Detailed cell-level brood development evaluations were made -2, 4, 8, 14 and 19 DAT; in each evaluation, digital images were taken of combs, and the content of individual cells (*i.e.*, empty, egg, young larvae [L1-L2], old larvae [L3-L5], pupae [capped cells], nectar, pollen, or dead) was color-coded by the NEXTREAT software. Brood termination rates were

calculated based on the failure of individual eggs or larvae to develop successfully. For calculation of the brood index and brood compensation index, the color-coded images for each assessment day were then compared to the bee brood development stage expected for each assessment day (process depicted **Figure 1**).

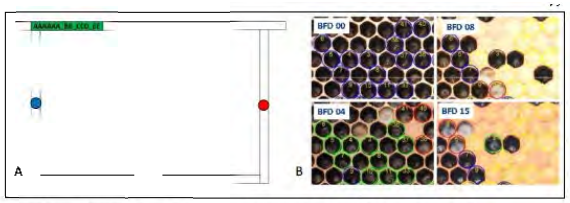

Figure 4: Detailed brood assessment with Nextreat digital image analysis tool

A) Schematic description of the initial comb labels; B) Schematic description of marking cells with the respective cell content using Nextreat tool

Table 6: Evaluation of detailed bee brood development (chronological test schedule)

Date of assessment*	Expected brood stage
BFD 0 (DAT -2)	Egg
BFD 6	Young larvae or old larvae
BFD 10	Pupae
BFD 16	Pupae
BFD 21	Empty cells or refilled after hatch

DAT = day after treatment; BFD = Brood area fixing day

Figure 1. Details on evaluation of bee brood development using NEXTREAT software (copied from registrant-submitted study report).

Test Environmental Conditions:

Ambient environmental conditions inside the tunnels (weather data for -4 to 7 DAT collected at an undescribed location at the test site; data for 8 to 27 DAT [data actually collected out to 93 DATs] acquired at the monitoring site) and reported here as daily means: 13.9-18.0 °C and 59.9-74.3% relative humidity (RH) before application; 19.4-20.4 °C and 48.9-56.2% RH during daytime applications, and 14.2-14.7 °C and 73.2-75.1% RH during night time applications; 12.7-17.2 °C and 58.2-84.5% RH during the 7-d exposure phase in the tunnels; and 14.6-25.9°C and 48.0-90.8% RH between 8 and 27 DATS at monitoring site. Rainfall (>1.0 mm) was reported during the study at 3, 7, 15, 16, 17, 18, 19, and 21 DAT and consisted of 7, 11.1, 55.0, 9.0, 6.5, and 3.0 mm, respectively.

Photoperiod and Lighting: Natural

Nominal and Measured Concentrations:

Negative control: tap water (400 L/ha)

Test item I (daytime application during bee flight): afidopyropen: 0.1 L/ha (10 g a.i./ha [nominal])
Reference item I – fenoxycarb: 300 g a.i./ha (nominal)
Reference item II - dimethoate: 480 g a.i./ha (nominal))

Test item II (evening application after bee flight): afidopyropen: 0.1 L/ha (10 g a.i./ha [nominal])

Test Plots:	The test site was located in Cunnersdorf near Leipzig, Germany. For the control and afidopyropen treatments (day and night applications), four separate tunnels (*i.e.*, replicates), were set up within a field of fully flowering *P. tanacetifolia*; three tunnels were used for the reference item I (fenoxycarb) treatments, and a single tunnel used for the reference item II (dimethoate) treatment. Each tunnel was 18 m length x 6 m width x 2.5 m height (108 m² floor space).
Test Design:	Tunnel test under semi-field conditions, with one bee hive per 108 m² tunnel. Tunnels were set up on a field of *P. tanacetifolia*, and healthy bee colonies were introduced on 17 June 2014, at BBCH development stage 63-65 (30-50% open flowers) of the crop, and five days before application (DAT -5). The application was carried out five days later during bee flight at full flowering of the crop (BBCH 65, full flowering). Bees were exposed to the water, afidopyropen and reference item (fenoxycarb or dimethoate)-treated phacelia in the tunnels for seven days. At 7 DAT, colonies were removed from the tunnels and relocated to a monitoring site approximately 5.5 km southeast. The monitoring site (near Brandis, Germany) was located in a forested area with no bee attractive crops.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration:	7 d
Endpoint(s):	Afidopyropen daytime application: increased adult mortality; decreased foraging activity; increased brood termination rate. Afidopyropen evening application: no effects
Effect Concentration:	Afidopyropen I: 0.1 L EP/ha Afidopyropen II: >0.1 L EP/ha
Basis for Concentration:	Nominal
Effect Concentration Type:	Test material
Basis for Effect:	Afidopyropen I (daytime application): increased adult mortality; decreased foraging activity; increased brood termination rate. Afidopyropen II (evening application): no effects

Applicant-Provided Results:

Application Conditions & Deviations: Applications were made using a single plot sprayer (Model PL 1, agrotop GmbH, Obertraubling, Germany) hand-held boom sprayer. Applications to the negative control, afidopyropen I and reference items I (fenoxycarb) & II (dimethoate) tunnels were made between 9:35 AM and 12:36 PM on 22 June 2014; applications to the afidopyropen II tunnels (evening) were made between 8:54 and 9:23 PM on 21 June 2014 (*i.e.* the evening of -1 DAT). Bee foraging activity prior to daytime applications was reported to be 12.0-15.0 bees/m^2 in study tunnels. Wind speed outside tunnels for all applications was 0.0-0.5 m/s. Mean temperature was 19.4-20.4 °C for daytime applications, and 14.2-14.7 °C for the evening application. Mean relative humidity was 48.9-56.2% for daytime applications, and 73.2-75.1% for the evening application. The amount of applied product (based on application volumes) deviated from the target application amount by -1.3 to 4.0% for afidopyropen applications, and 2.4 to 7.2% for the fenoxycarb and dimethoate applications.

Sublethal Behavioral Effects: Sublethal behavioral effects were apparently only observed and recorded for afidopyropen treatments (and for these treatments only for 0-7 DAT), and not the negative control or fenoxycarb/dimethoate reference item treatments. According to the study authors, there were no reported observations of sublethal behavioral effects in evening afidopyropen application (test item II) tunnels; however, there were sublethal effects in bees in the daytime afidopyropen application tunnels. In these tunnels, within 30 minutes of applications 10-30 bees in each tunnel were motionless, showed reduced ability to respond to stimulation, fell off of crop plants, exhibited impaired locomotion and/or cramping. These sublethal effects were reported to have occurred only through the end of 0 DAT, by even one day after applications the previously observed sublethal effects were no longer apparent in test item colonies.

Adult & Juvenile Mortality: According to the study author, there were no statistically significant differences in adult worker bee mortality between the negative control colonies and either the colonies from afidopyropen-treated (I or II) tunnels or the reference item colonies (fenoxycarb or dimethoate) (see **Table 1**). Apparently, on the day of application following treatment (*i.e.*, 0aa DAT) and 1 DAT, adult mortality in colonies that received daytime afidopyropen applications was significantly different (139% higher; $p < 0.05$) than in negative control colonies.

According to the study author, during the exposure and monitoring phases, no dead pupae were found in negative control or afidopyropen (I or II) colonies; therefore, the study author did not perform statistical analyses on pupal mortality data.

Table 1. Study author-reported effects on bee (*Apis mellifera*) mortality, foraging activity, and bee brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for control, formulated afidopyropen (BAS 440 00 I (9.8% active ingredient)-treated, and dimethoate or fenoxycarb (reference)-treated colonies (means ± standard deviation are reported [except for dimethoate]).

	Control		Afidopyropen		Fenoxycarb [1]	Dimethoate [2]
	Daytime	Evening	Daytime	Evening		
Mean mortality of adult worker bees (n dead bees/colony/day)						
Pre-application phase [3, 4]	26.1 ± 5.1	29.9 ± 6.3	25.9 ± 5.9	29.1 ± 9.4	31.4 ± 5.5	35.0
Exposure phase in the tunnels [3,5]	29.3 ± 5.1	30.7 ± 5.1	30.8 ± 5.0	31.1 ± 10.3	29.5 ± 9.3	244.5
Monitoring phase outside the tunnels [6]	13.4 ± 2.3		11.4 ± 2.2	16.3 ± 2.2	11.3 ± 1.5	17.5
Mean mortality of pupae (n dead pupae/colony/day) [7]						
Pre-application phase	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Exposure phase in the tunnels	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Monitoring phase outside the tunnels	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0	16.8 ± 6.7	0.0
Mean foraging activity/m^2/colony/day [n]						
Pre-application phase	12.2 ± 0.6	11.9 ± 0.7	12.4 ± 0.9	12.4 ± 0.5	12.0 ± 0.2	12.7
Exposure phase in the	11.5 ± 0.5	11.6 ± 0.5	11.4 ± 0.6	13.0 ± 0.4	13.5 ± 0.2	0.4

tunnels						

1) Mean value of three replicate tunnels.
2) Value represents data collected from a single tunnel, so no standard deviation is calculated.
3) Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
4) Control means related to 'daytime' afidopyropen applications represent an average across the following assessments: -4 to 0ba DAT ('ba' assessment made on the day of applications, but immediately before applications). Control means related to 'evening' afidopyropen applications represent an average across the following assessments: -4 to -1ba DAT (assessment made -1 DAT prior to the evening application of the test item).
5) Control means related to 'daytime' afidopyropen applications represent an average across the following assessments: 0aa to 7 DAT ('aa' assessment made on the day of applications, but immediately after applications). Control means related to 'evening' afidopyropen applications represent an average across the following assessments: 0 to 7 DAT.
6) Mean number of dead honeybees per day and colony found in dead bee traps, only.
7) Data on mean mortality of pupae were not statistically analyzed by the study author.

DAT = days after treatment

Foraging Activity: According to the study authors, there were no statistically significant differences in mean foraging activity between colonies from the negative control and either the afidopyropen-treated tunnels (I or II) or the reference item colonies (fenoxycarb or dimethoate) (see **Table 1**). Apparently, mean foraging behavior in colonies treated with afidopyropen during the daytime and in colonies treated with dimethoate decreased within one hour of applications compared to the control, and remained depressed until the following day, after which mean foraging activity in these colonies seemed to equalize relative to control colonies.

Colony Strength: The study author did not appear to statistically analyze colony strength (estimated number of bees per colony) data, but nevertheless stated that there was no indication of adverse effects from either afidopyropen treatment on overall colony strength (see **Table 2**). On the other hand, according to the study author, both reference item (fenoxycarb and dimethoate) treatments reduced colony strength over the duration of the study relative to initial values (*i.e.* -2 DAT).

Table 2. Summary of colony strength (mean number of worker bees) in control, afidopyropen (test item I & II) and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Colony strength [estimated average number of bees/colony]								
	Control			Test item I (day application)			Test item (evening application)		
	Mean[1]	± SD	%[2]	Mean[1]	± SD	%[2]	Mean[1]	± SD	%[2]
BFD 0 (DAT -2)	9563	1222	-	9141	425	-	9984	834	-
BFD 6 (DAT 4)	10238	559	+7	9759	232	+7	10378	731	+4
BFD 10 (DAT 8)	11559	1170	+21	11363	595	+24	11363	1293	+14
BFD 16 (DAT 14)	12234	1499	+28	12347	598	+35	12122	1254	+21
BFD 21 (DAT 19)	11447	878	+20	10322	839	+13	11166	1554	+12
BFD 28 (DAT 26)	12375	543	+29	11869	436	+30	10913	2353	+9
BFD 71 (DAT 69)	14259	684	+49	14344	563	+57	15159	563	+52
BFD 95 (DAT 93)	13894	864	+45	13106	854	+43	13416	854	+34
Assessment day	**Control**			**Reference item I (Fenoxycarb)**			**Reference item II (Dimethoate)**		
	Mean[1]	± SD	%[2]	Mean[3]	± SD	%[2]	Mean[4]	± SD	%[2]
BFD 0 (DAT -2)	9563	1222	-	10238	849	-	11363	-	-
BFD 6 (DAT 4)	10238	559	+7	11138	338	+9	9225	-	-19
BFD 10 (DAT 8)	11559	1170	+21	10725	732	+5	10125	-	-11
BFD 16 (DAT 14)	12234	1499	+28	9938	844	-3	9563	-	-16
BFD 21 (DAT 19)	11447	878	+20	7500	520	-27	7650	-	-33
BFD 28 (DAT 26)	12375	543	+29	6750	1073	-34	7425	-	-35
BFD 71 (DAT 69)	14259	684	+49	6600	1149	-36	8775	-	-23
BFD 95 (DAT 93)	13894	864	+45	6338	1690	-38	9563	-	-16

DAT: day after treatment
BFD: Brood area fixing day
[1] mean of four replicates
[3] mean of three replicates
[4] one replicate
[2] relative change in comparison with BFD 0 (DAT -2) calculated from the respective mean values

Colony Condition: According to the study authors, overall, the applications of afidopyropen during daytime resulted in slight and temporary adverse effects on brood condition (*i.e.*, estimated brood area occupied by eggs), but had no adverse effect on brood development over time (see **Table 3**). The study author reported that the estimated comb area covered with food stores (nectar, honey and pollen) was similar in control colonies, afidopyropen (I & II) colonies, and in fenoxycarb colonies, but was slightly higher (relative to controls) in dimethoate colonies (see **Table 4**). Furthermore, the study author reported that during the exposure phase the estimated comb area occupied by pollen increased across treatments.

Table 3. Summary of brood strength (estimated brood area per colony) in control, afidopyropen (test item I & II) and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced with minor edits from applicant-submitted study report.

	Estimated brood area per colony1F[2] [cm²/colony]								
	Treatment group								
	Control			Test item I (day application)			Test item II (evening application)		
BFD	Mean#	SD	% to BFD 0*	Mean#	SD	% to BFD 0*	Mean#	SD	% to BFD 0*
	Eggs + Larvae + Pupae								
0	8328	889	-	8045	1055	-	8071	1224	-
6	7980	1264	-4	7258	501	-10	7013	879	-13
10	6781	638	-19	5569	629	-31	6446	917	-20
16	7735	601	-7	6395	1042	-21	6446	536	-20
21	8406	756	+1	6407	387	-20	6343	1080	-21
28	9205	1089	+11	7400	341	-8	8019	1233	-1
71	4564	889	-45	4151	706	-48	4564	1106	-43
95	3146	1138	-62	2682	404	-67	3507	1537	-57
	Control			Reference item I (Fenoxycarb)			Reference item II (dimethoate)		
BFD	Mean#	SD	% to BFD 0*	Mean##	SD	% to BFD 0*	Mean###	SD	% to BFD 0*
	Eggs + Larvae + Pupae								
0	8328	889	-	8595	746	-	8457	-	-
6	7980	1264	-4	5569	1364	-35	4435	-	-48
10	6781	638	-19	3507	676	-59	3146	-	-63
16	7735	601	-7	3335	671	-61	3507	-	-59
21	8406	756	+1	3747	315	-56	3919	-	-54
28	9205	1089	+11	3885	760	-55	5569	-	-34
71	4564	889	-45	3472	1195	-60	3507	-	-59
95	3146	1138	-62	2475	516	-71	2372	-	-72

BFD: Brood area fixing day # mean of four replicates ## mean of three replicates ### one replicate * relative change in comparison with DAT -2 calculated from the respective mean values

Table 4. Summary of food stores (nectar, honey and pollen) in control, afidopyropen (test item I & II) and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced with minor edits from applicant-submitted study report.

Estimated food area per colony3F[4] [cm²/colony]									
	Treatment group								
	Control			Test item I (day application)			Test item II (evening application)		
BFD	Mean[a]	SD	% to BFD 0*	Mean[a]	SD	% to BFD 0*	Mean[a]	SD	% to BFD 0*
	Entire food (nectar + pollen)								
0	3300	855	-	2991	739	-	3687	852	-
6	4151	1657	+26	4641	767	+55	4822	980	+31
10	4280	1279	+30	5247	596	+75	6678	2594	+81
16	5698	1789	+73	6627	638	+122	6833	1026	+85
21	6059	818	+84	6652	783	+122	8277	1469	+124
28	6807	1345	+106	7555	1083	+153	7838	1984	+113
71	10623	1313	+222	10443	1076	+249	10984	1096	+198
95	17817	2336	+440	17611	1704	+489	17404	2815	+372
	Control			Reference item I (Fenoxycarb)			Reference item II (dimethoate)		
BFD	Mean[a]	SD	% to BFD 0*	Mean[aa]	SD	% to BFD 0*	Mean[aaa]	SD	% to BFD 0*
	Entire food (nectar + pollen)								
0	3300	855	-	3575	958	-	4641		-
6	4151	1657	+26	5844	604	+63	5363		+16
10	4280	1279	+30	6463	1708	+81	6291		+36
16	5698	1789	+73	7598	1150	+113	6498		+40
21	6059	818	+84	7048	1857	+97	6498		+40
28	6807	1345	+106	6326	1579	+77	6498		+40
71	10623	1313	+222	5398	936	+51	7426		+60
95	17817	2336	+440	11345	3400	+217	14130		+204

BFD: Brood area fixing day [a] mean of four replicates [aa] mean of three replicates [aaa] one replicate * relative change in comparison with DAT -2 calculated from the respective mean values

Brood Development Indices: According to the study author, the mean brood index and brood compensation index were significantly ($p < 0.05$) lower in colonies that received a daytime application of afidopyropen relative to control colonies, but there were no significant differences in mean index values in colonies receiving the evening afidopyropen application relative to control colonies (see **Table 5**). Likewise, the mean brood termination rate in daytime afidopyropen colonies was significantly ($p < 0.05$) higher than in control colonies, and was equivalent in colonies receiving the evening afidopyropen application. Similar responses (*i.e.*, significantly [$p < 0.05$] lower mean brood index and brood compensation index, and higher mean brood termination rate) were also reported for colonies receiving the fenoxycarb applications relative to the control colonies.

Table 5. Summary of brood development indices (brood index, brood compensation index, and brood termination rate) in control, afidopyropen (test item I & II) and fenoxycarb colonies at specified days after treatment (DAT). Table reproduced with minor edits from applicant-submitted study report.

Assessment day (BFD)	Mean brood index of initially labelled eggs [%]							
	Treatment group							
	Control		Test item I (day application)		Test item II (evening application)		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
06	2.8	0.6	1.9*	0.3	1.8	1.2	0.01*	0.01
10	3.2	0.6	2.0*	0.3	2.2	1.5	0.01*	0.01
16	3.4	0.5	2.0*	0.2	2.2	1.4	0.00*	0.01
21	3.9	0.8	2.4*	0.3	2.8	1.8	0.01*	0.01
Assessment day (BFD)	**Mean brood compensation index of initially labelled eggs [%]**							
	Treatment group							
	Control		Test item I (day application)		Test item II (evening application)		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
06	2.9	0.6	1.9*	0.3	1.9	1.1	0.05*	0.07
10	3.2	0.5	2.0*	0.3	2.3	1.4	0.01*	0.01
16	3.5	0.3	2.0*	0.3	2.4	1.3	0.08*	0.14
21	4.1	0.6	2.9*	0.2	3.1	1.5	0.41*	0.34
Assessment day (BFD)	**Mean brood termination rate of initially labelled eggs [%]**							
	Treatment group							
	Control		Test item I (day application)		Test item II (evening application)		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
06	17.9	14.5	48.1*	8.3	42.9	37.2	99.8*	0.2
10	21.8	15.4	49.9*	6.7	44.6	35.9	99.8*	0.2
16	22.1	14.8	51.4*	5.9	44.8	36.0	99.9*	0.2
21	22.1	15.8	51.4*	5.9	44.8	36.0	99.9*	0.2

BFD: Brood area fixing day; [1] mean of four replicates; [2] mean of three replicates;
* = statistically significantly different (STUDENT t-test) one-sided greater, p<0.05.

Residues: The study author reported that no afidopyropen residues were found in flower, leaf, nectar or pollen specimens collected at random locations in control or test item (I or II) tunnels before applications were made; additionally, no residues were reportedly found in specimens collected in negative control treatment tunnels following applications. Immediately following (<4 h) applications afidopyropen residues in *Phacelia* flowers were 1.45 and 1.76 mg a.i./kg, respectively, in samples collected from tunnels receiving the daytime and evening test item applications. Afidopyropen residues in pollen were 0.47 and 0.19 mg a.i./kg, respectively, in samples collected from tunnels receiving the daytime and evening test item applications. Afidopyropen residues in nectar were less than the limit of quantification (<LOQ of 0.01) and 0.03 mg a.i./kg, respectively, in samples collected from tunnels receiving the daytime and evening test item applications.

Weather Data: Weather data reported by the study author is summarized in **Figure 2**, and includes total daily precipitation (mm), daily mean temperature (°C), daily mean humidity (% RH), and cloud cover (%) for the pre-application and exposure phases of the study. The study author noted that during the pre-

application phase of the study mean daily temperatures ranged between 14 to18°C; while there was minimal precipitation, cloud cover was 100% -3 to -1 DATs. During the exposure phase of the study cloud cover was more moderate, but daily minimum temperatures were below 10 °C on 1, 2, 4 and 5 DATs, there was substantial rainfall at 3 (20 mm) and 7 DATs (8 mm). During the monitoring phase of the study, daily minimum temperatures were below 10 °C on 10 and 11 DATs, and daily maximum temperatures exceeded 30 °C on 12, 14, 26 and 27 DATs; there was 55 mm of rainfall 16 DAT, and 11, 9, 6.5, 2 and 2 mm of precipitation, respectively, 15, 17, 18, 21 and 26 DATs.

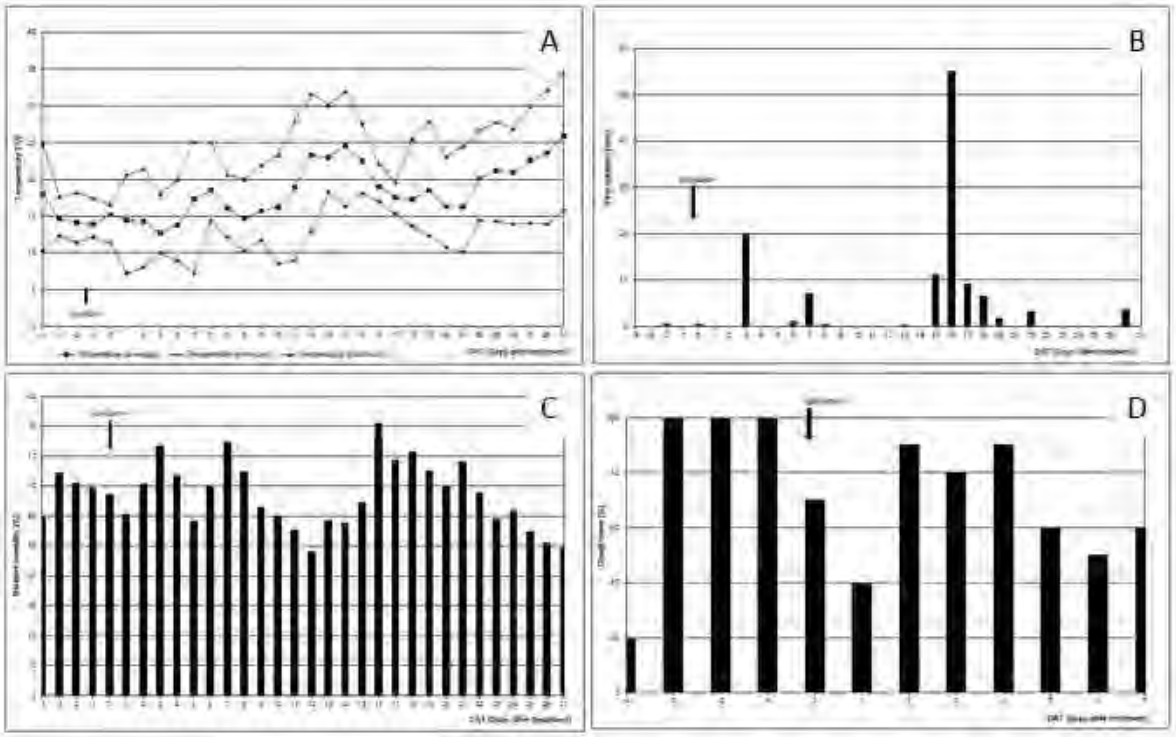

Figure 2. Summary of study author-provided data on daily temperature ('A'), precipitation ('B'), relative humidity ('C'), and cloud cover ('D').

Overall, the study author concluded that applications of BAS 440 00 I during bee flight (*i.e.*, during the daytime) resulted in some effects on brood development, and transient effects on worker bee mortality, but that applications of BAS 440 00 I in the absence of bee flight (*i.e.*, in the evening) did not adversely affect honeybee colonies in this study.

Applicant-Reported Statistics and Error Estimates

The applicant reported means and standard deviations for all endpoints, included calculated brood indices; the following endpoints were statistically analyzed by the study author: adult worker bee mortality; foraging activity; brood index; brood compensation index; and, brood termination rate. Easy Assay 4.0 and ToxRat Professional (ver. 3.0 beta) were used for all of the study author's statistical analyses.

Data were apparently tested for the homogeneity of variances per the study author's descriptions of statistical methods in the study report, but it is not clear what test was used for the comparison of variances, and it's not stated whether the distribution of data were tested for normality. Pre-treatment data were statistically evaluated using a Tukey's Test, and post-treatment data were statistically evaluated using pairwise Student t-tests or Welch t-test for comparisons versus the control. All pre-application comparisons were made using two-sided tests, and all post-application comparisons were made using one-sided tests (*i.e.*, "greater" for mortality and brood termination rate, and "smaller" for foraging activity, brood index and brood compensation index).

IV. OVERALL REMARKS, ATTACHMENTS

Microsoft Excel data tables were submitted with an OECD-formatted summary by the registrant. The applicant did not include raw data on measured residues in the provided Excel tables, and so these data were manually extracted from the study report by the reviewer.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

The reviewer verified all of the applicant's calculations (where possible – see following note) and carried out statistical analyses per relevant EFED guidance for all data to confirm the applicant's results and conclusions. The study author provided only summary data for the detailed (cell-level) evaluation of brood development indices (brood index, brood compensation index, and brood termination rate), as such it was not possible for the reviewer to thoroughly verify the study author's calculations of replicate-level brood development indices. Replicate-level means for these data were extracted by the reviewer from the study report and used to confirm statistical conclusions.

Adult & Juvenile Mortality: There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen daytime or evening applications or fenoxycarb treatment groups and the negative control during the pre-application or exposure phases of the study (**Table 6**). Worker bee mortality during the exposure phase in the single dimethoate tunnel (177.82 dead bees/colony/d) was higher than mean worker bee mortality in the control tunnels (21.85-22.86 dead bees/colony/d), but this difference could not be statistically tested. During the monitoring phase, mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, lower) in daytime application afidopyropen tunnels (15%) and in fenoxycarb-treated tunnels (16%) compared to control tunnels and was not considered an adverse effect; during the same phase, mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, 21% higher) in evening application afidopyropen tunnels compared to control tunnels. Worker bee mortality during the monitoring phase in the single dimethoate tunnel (17.50 dead bees/colony/d) was higher than mean worker bee mortality in the control tunnels (13.38 dead bees/colony/d), but this difference could not be statistically tested.

Data on mean mortality of pupae were not analyzed statistically by the reviewer due to measurable mortality (per reported data) only occurring in a single treatment group at a single point in the study (*i.e.*, mean: 16.80% pupal mortality in fenoxycarb-treated colonies during the monitoring phase of the study). Reported mortality of pupae in all other treatment groups at all other time points in the study was 0% (**Table 6**).

Table 6. Reviewer-calculated effects on bee (*Apis mellifera*) mortality, foraging activity, and bee brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for control, formulated afidopyropen (BAS 440 00 I; 9.8% active

ingredient)-treated, and dimethoate or fenoxycarb (reference)-treated colonies (means ± standard deviation are reported [except for dimethoate]).

	Control		Afidopyropen		Fenoxycarb [1]	Dimethoate [2]
	Daytime	Evening	Daytime	Evening		
Mean mortality of adult worker bees (n dead bees/colony/day)						
Pre-application phase [3, 4]	21.71 ± 2.78	23.90 ± 3.12	21.58 ± 2.43	23.30 ± 3.19	26.17 ± 3.82	29.17
Exposure phase in the tunnels [3,5]	22.86 ± 2.25	21.85 ± 2.12	22.41 ± 2.32	21.46 ± 2.36	22.76 ± 2.68	177.82
Monitoring phase outside the tunnels [6]	13.38 ± 0.65		11.35 ± 0.61†	16.21 ± 0.81†	11.28 ± 0.91†	17.50
Mean mortality of pupae (n dead pupae/colony/day) [7]						
Pre-application phase	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Exposure phase in the tunnels	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Monitoring phase outside the tunnels	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0	16.80 ± 1.98	0.0
Mean foraging activity/m²/colony/day [n]						
Pre-application phase	12.22 ± 0.39	11.85 ± 0.46	12.42 ± 0.36	12.35 ± 0.41	12.02 ± 0.39	12.67
Exposure phase in the tunnels	12.67 ± 0.16	12.72 ± 0.15	9.29 ± 0.39†	13.03 ± 0.28†	13.52 ± 0.30†	1.33

1) Mean value of three replicate tunnels.
2) Value represents data collected from a single tunnel, so no standard deviation is calculated; consequently, this treatment group was excluded from all statistical analyses.
3) Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
4) Control means related to 'daytime' afidopyropen applications represent an average across the following assessments: -4 to 0ba DAT ('ba' assessment made on the day of applications, but immediately before applications). Control means related to 'evening' afidopyropen applications represent an average across the following assessments: -4 to -1ba DAT (assessment made -1 DAT prior to the evening application of the test item).
5) Control means related to 'daytime' afidopyropen applications represent an average across the following assessments: 0aa to 7 DAT ('aa' assessment made on the day of applications, but immediately after applications). Control means related to 'evening' afidopyropen applications represent an average across the following assessments: 0 to 7 DAT.
6) Mean number of dead honeybees per day and colony found in dead bee traps, only.
7) Data on mean mortality of pupae were not statistically analyzed by the reviewer.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's t test
† = statistically significant differences ($p < 0.05$) compared to the control, pairwise Mann-Whitney test
DAT = days after treatment

Foraging Activity: There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen (daytime or evening applications) or fenoxycarb treatment groups and the negative control during the pre-application phase of the study (**Table 6**). During the exposure phase of the study, mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 27% lower) in daytime application afidopyropen tunnels, and was significantly ($p < 0.05$) different (*i.e.*, higher) in evening application afidopyropen tunnels (2% higher) and in fenoxycarb-treated tunnels (7% higher); foraging activity in the single dimethoate-tunnel was 90% lower than in control tunnels, but this difference could not be statistically tested.

Colony Strength: The mean number of adult worker bees in afidopyropen-treated tunnels (both daytime and evening applications) was equivalent to that in control tunnels throughout the study (**Table 7**). At

19, 26, 69, and 93 DATs the mean number of worker bees in fenoxycarb-treated tunnels was significantly ($p<0.05$) different (*i.e.*, lower by 39-54%) than the mean number of worker bees in the control tunnels. The mean number of adult worker bees in the single dimethoate-treated tunnel was similarly lower (32-40%) than in control tunnels during the monitoring phase of the study, but this difference could not be statistically tested.

The mean number of pupae in afidopyropen-treated tunnels (both daytime and evening applications) was equivalent to that in control tunnels throughout the study, except for 19 DAT, when the mean number of pupae was significantly ($p<0.05$) different (*i.e.*, lower by 37-39%) than the mean number of pupae in the control tunnels (**Table 7**). At 8, 14, 19, and 26 DATs the mean number of pupae in fenoxycarb-treated tunnels was significantly ($p<0.05$) different (*i.e.*, lower by 41-92%) than the mean number of worker bees in the control tunnels. The mean number of adult worker bees in the single dimethoate-treated tunnel was similarly lower (35-86%) than in control tunnels during the monitoring phase of the study, but this difference could not be statistically tested.

Colony Condition: The mean number of brood (eggs, larvae and male brood) cells in afidopyropen-treated tunnels (both daytime and evening applications) was equivalent to that in control tunnels throughout the study (**Table 7**). At 4, 8, and 26 DATs the mean number of brood cells in fenoxycarb-treated tunnels was significantly ($p<0.05$) different (*i.e.*, lower by 59-70%) than the mean number of brood cells in the control tunnels. The mean number of brood cells in the single dimethoate-treated tunnel was similarly lower (46-62%) than in control tunnels during the monitoring phase of the study, but this difference could not be statistically tested.

The mean number of food (nectar and pollen) cells in afidopyropen-treated tunnels (both daytime and evening applications) and in reference item tunnels (both fenoxycarb and dimethoate) was equivalent to that in control tunnels throughout the study (**Table 7**).

Table 7. Reviewer-calculated effects on honey bee (*Apis mellifera*) colony strength and condition under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb or dimethoate-treated colonies (means ± standard error are reported).

	Days After Treatment (DAT)							
	-2	**4**	**8**	**14**	**19**	**26**	**69**	**93**
Colony Strength – Adults (est. n adult bees/colony/d)								
Control	9,563 ± 611	10,238 ± 279	11,560 ± 585	12,235 ± 750	11,447 ± 439	12,375 ± 272	14,260 ± 342	13,894 ± 432
Afido. I [1]	9,141 ± 212	9,760 ± 116	11,363 ± 298	12,347 ± 299	10,322 ± 419	11,869 ± 218	14,344 ± 281	13,107 ± 427
Afido. II [2]	9,985 ± 417	10,379 ± 366	11,363 ± 646	12,122 ± 627	11,166 ± 777	10,913 ± 1,176	15,160 ± 1,159	13,416 ± 1,255
Fenoxycarb [3]	10,238 ± 490	11,138 ± 195	10,725 ± 422	9,938 ± 487	**7,500 ± 300***	**6,750 ± 620***	**6,600 ± 263***	**6,338 ± 358***
Dimethoate [4]	11,363	9,225	10,125	9,563	7,650	7,425	8,775	9,563
Colony Strength – Juveniles (est. n pupae/colony/d)								
Control	5,766 ± 202	5,245 ± 473	4,444 ± 463	3,966 ± 645	4,753 ± 261	6,047 ± 536	1,913 ± 189	2,419 ± 402
Afido. I [1]	5,541 ± 377	5,470 ± 476	3,923 ± 371	3,066 ± 385	**2,911 ± 135***	5,203 ± 323	1,856 ± 281	1,997 ± 106

Afido. II [2]	5,878 ± 606	5,316 ± 480	4,641 ± 422	3,234 ± 552	**3,009 ± 281***	5,709 ± 635	2,475 ± 350	2,419 ± 548
Fenoxycarb [3]	5,700 ± 423	5,025 ± 922	**2,625 ± 442***	**300 ± 188***	**388 ± 225***	**2,700 ± 455***	1,613 ± 163	1,800 ± 172
Dimethoate [4]	5,625	3,263	2,306	563	900	3,938	2,250	1,013
Colony Condition – Brood (est. n cells/colony/d as brood)								
Control	1,659 ± 242	1,730 ± 303	1,477 ± 152	2,236 ± 202	2,208 ± 289	1,997 ± 251	1,533 ± 204	506 ± 122
Afido. I [1]	1,617 ± 218	1,223 ± 94	1,076 ± 167	1,955 ± 196	2,039 ± 233	1,434 ± 156	1,336 ± 117	464 ± 119
Afido. II [2]	1,463 ± 205	1,167 ± 174	1,195 ± 122	1,898 ± 218	1,955 ± 261	1,519 ± 149	1,252 ± 119	703 ± 156
Fenoxycarb [3]	1,838 ± 188	**525 ± 246***	**600 ± 269***	1,669 ± 216	1,650 ± 135	**769 ± 210***	1,088 ± 386	450 ± 96
Dimethoate [4]	1,800	788	563	1,631	1,688	1,069	788	788
Colony Condition – Food (est. n cells/colony/d as food)								
Control	1,800 ± 447	2,264 ± 639	2,334 ± 701	3,108 ± 1018	3,305 ± 1051	3,713 ± 671	5,794 ± 1612	9,717 ± 3389
Afido. I [1]	1,631 ± 445	2,531 ± 656	2,862 ± 790	3,558 ± 1144	3,628 ± 1220	4,120 ± 826	5,695 ± 1743	9,605 ± 3427
Afido. II [2]	2,011 ± 582	2,630 ± 684	3,642 ± 1,158	3,727 ± 1,094	4,514 ± 1,416	4,275 ± 785	5,991 ± 1,567	9,492 ± 3,230
Fenoxycarb [3]	1,950 ± 555	3,188 ± 604	3,525 ± 955	4,106 ± 1261	3,844 ± 1086	3,450 ± 657	2,869 ± 893	6,188 ± 2880
Dimethoate [4]	2,531	2,925	3,431	3,544	3,544	3,544	4,050	7,706

[1)] Refers to test item I treatment group, which was treated during the daytime when honeybees were in full flight.
[2)] Refers to test item II treatment group, what was treated in the evening when no bees were foraging.
[3)] Mean value of three replicate tunnels.
[4)] Value represents data collected from a single tunnel, so no standard error is calculated for colony strength endpoint; consequently, this treatment group is excluded from all statistical analyses.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test

Brood Development Indices: The mean brood index and brood compensation index was significantly ($p<0.05$) different (*i.e.*, lower by 35-38 and 29-44%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies (**Tables 8 & 9**). The mean brood termination rate was significantly ($p<0.05$) different (*i.e.*, higher by 130-169%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies.

Overall effects from evening applications of afidopyropen were similar to effects from daytime applications, though of slightly lower magnitude – *i.e.*, lower brood index and brood compensation index, and higher brood termination rate – but these effects were not statistically significantly different from those in control colonies due to higher variance around treatment means (**Table 9** [see "Reviewer's Statistical Verification" for further discussion]).

In general, fenoxycarb treatments appeared to result in very low mean brood index (0.00 - 0.01), very low mean brood compensation index (0.01-0.38), and very high mean brood termination rates (99.78-99.89%). While these treatment responses are biologically relevant, they could not be statistically compared to the negative control due to issues discussed further in the next section of this document ("Reviewer's Statistical Verification").

Table 8. Reviewer-calculated effects on honey bee (*Apis mellifera*) brood development indices under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb-treated colonies (means ± standard error are reported).

	Days After Treatment (DAT)			
	4	**8**	**14**	**19**
Brood Index (bi)				
Control	2.83 ± 0.30	3.15 ± 0.29	3.15 ± 0.29	3.90 ± 0.39
Afidopyropen I [1]	1.85 ± 0.16 ¶	2.00 ± 0.14 ¶	1.94 ± 0.12 ¶	2.42 ± 0.15 ¶
Afidopyropen II [2]	1.83 ± 0.57	2.23 ± 0.73	2.21 ± 0.72	2.75 ± 0.89
Fenoxycarb [3]	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Brood Compensation Index (bci)				
Control	2.85 ± 0.28	3.20 ± 0.27	3.55 ± 0.13	4.06 ± 0.32
Afidopyropen I [1]	1.86 ± 0.17 ¶	2.02 ± 0.14 ¶	1.99 ± 0.14 ¶	2.90 ± 0.11 ¶
Afidopyropen II [2]	1.86 ± 0.56	2.27 ± 0.72	2.44 ± 0.65	3.14 ± 0.78
Fenoxycarb [3]	0.05 ± 0.04	0.01 ± 0.00	0.08 ± 0.08	0.38 ± 0.22
Brood Termination Rate (btr, %)				
Control	17.91 ± 7.71	21.75 ± 7.70	22.07 ± 7.88	22.07 ± 7.88
Afidopyropen I [1]	48.08 ± 4.13 ¶	49.91 ± 3.36 ¶	51.41 ± 2.94 ¶	51.41 ± 2.94 ¶
Afidopyropen II [2]	42.91 ± 18.58	44.59 ± 17.94	44.75 ± 18.01	44.75 ± 18.01
Fenoxycarb [3]	99.78 ± 0.11	99.78 ± 0.11	99.89 ± 0.11	99.89 ± 0.11

[1)] Refers to test item I treatment group, which was treated during the daytime when honeybees were in full flight.

[2)] Refers to test item II treatment group, what was treated in the evening when no bees were foraging.

[3)] Mean value of three replicate tunnels; this treatment group was excluded from statistical analyses due to issues discussed in the "Reviewer's Statistical Verification" section of this document.

¶ = statistically significant differences ($p < 0.05$) compared to the control, Welch's t-test

Table 9. Reviewer-calculated summary of mean effect (% relative to control) and variance of mean effects on honey bee (*Apis mellifera*) brood development indices under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb-treated colonies.

	Mean Effect (% relative to control)				Variance (s^2)			
	4	**8**	**14**	**19**	**4**	**8**	**14**	**19**
Brood Index (bi)								
Control	N/A				0.36	0.34	0.35	0.61
Afidopyropen I [1]	-34.6	-36.5	-38.4	-37.9	0.47	0.55	0.59	0.92
Afidopyropen II [2]	-35.3	-29.2	-29.8	-29.5	1.00	1.30	1.29	2.01
Fenoxycarb [3]	-99.6	-99.7	-100.0	-99.7	<0.01	<0.01	<0.01	<0.01
Brood Compensation Index (bci)								
Control	N/A				0.31	0.29	0.06	0.40
Afidopyropen I [1]	-34.7	-36.9	-43.9	-28.6	0.46	0.56	0.76	0.58
Afidopyropen II [2]	-34.7	-29.1	-31.3	-22.7	0.95	1.27	1.11	1.45
Fenoxycarb [3]	-98.2	-99.7	-97.7	-90.6	<0.01	<0.01	0.02	0.14
Brood Termination Rate (btr, %)								
Control	N/A				237.47	237.33	248.36	248.37
Afidopyropen I [1]	+168.5	+129.5	+132.9	+132.9	391.22	347.68	367.37	367.37
Afidopyropen II [2]	+139.6	+105.0	+102.8	+102.8	872.01	802.21	809.62	809.62
Fenoxycarb [3]	+457.1	+358.8	+352.6	+352.6	0.04	0.04	0.04	0.04

Residues: Note that for analysis of afidopyropen residues in relevant matrices (*i.e.*, flowers, leaves, nectar and pollen) a single pooled sample was collected from the separate residue sampling-only afidopyropen tunnel, so no statistical analyses could be carried out on reported residue results for these data. Please reference Section III above for the study author's reported residue results.

Reviewer's Statistical Verification:
Statistical analyses confirmed using R (ver. 3.2.5)[2] statistical software, and the multcomp[3] analysis package. The reviewer relied on the Shapiro-Wilk's test and Bartlett's test to evaluate whether data were normally distributed or homoscedastic, respectively. ANOVA and Dunnett's multiple means test was used to test for statistical differences amongst means for data that met assumptions for parametric tests (*i.e.,* data were approximately normally distributed and had homogenous variances), and Kruskal-Wallis and Wilcoxon Rank Sum test was used for non-parametric means comparisons. One-sided tests were used for all hypothesis-based testing; $\alpha = 0.05$ for all mean comparison tests, and $\alpha = 0.01$ for all assumptions testing.

The brood development indices datasets were a statistical challenge as the full datasets (containing the negative control, afidopyropen I & II, and fenoxycarb tunnel data) were not approximately normally distributed, and exhibited unequal variances around the treatment mean (see **Appendix I** and **Table 9**). The distributions of the datasets were particularly sensitive to very large differences in responses of the fenoxycarb-treated colonies (for all three brood development indices) relative to both the negative control and afidopyropen-treated colonies. The mean brood index and brood compensation index for fenoxycarb-treated colonies were both low (<0.01 – 0.14) relative to the negative control, and the mean brood termination rate for fenoxycarb-treated colonies was high (>99%) relative to control and to the other treatment groups. In addition to very different responses (*i.e.*, treatment effects), variances around the mean for fenoxycarb-treated tunnels was low relative to variances around the mean for the other treatment groups (**Table 9**). To facilitate statistical analyses and focus on treatment responses due to afidopyropen applications the EFED reviewer analyzed brood development indices data without including the fenoxycarb tunnel data. In doing so, the resulting dataset (which included data only from the negative control and afidopyropen tunnels) was approximately normally distributed (see **Appendix I**), which allowed for the comparison of individual afidopyropen group means against the negative control treatment mean using Welch's t-tests (which are relatively insensitive to unequal variances around the treatment mean).

See **Appendix I** for summary statistics and diagnostic tests (*i.e.,* goodness-of-fit and equivalent variances tests) for all data described in this data evaluation report.

Based on statistically significant adverse effects on adult worker honeybee mortality, foraging activity, and brood development in colonies receiving daytime afidopyropen applications, the no-observed adverse effect level (NOAEL) across the various measurement endpoints for is <10 g a.i./ha under the conditions tested for this treatment.

[2] R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
[3] Hothorn T, F Bretz and P Westfall. 2008. Simultaneous inference in general parametric models. Biometric Journal 50: 346-363.

Reviewer's Comments:
The reviewer's overall results and conclusions agreed with those of the study author, and in spite of some differences regarding approaches towards statistically analyzing the study data, the reviewer and the study author agreed on the significance of treatment responses for particular endpoints. The study author did not statistically analyze colony strength or condition data, so comparisons between the reviewer's and study author's conclusions for these endpoints is not possible.

In terms of statistical approaches, the study author claimed in the study report that data were tested to see whether they met assumptions of parametric tests, and the statistical tests used by the author are all parametric tests. However, the reviewer's analysis indicated that all of the datasets analyzed by the study author did not met assumptions for parametric tests, and should have been analyzed using non-parametric tests. Ultimately, the study author's approach to statistically analyzing the datasets resulted in the same overall conclusions as the reviewer's, likely in part due to the study author's reliance on t-tests to analyze all of the post-treatment (which are relatively less sensitive to deviations from normality).

Data provided in the study report indicate that the average time to make applications to each tunnel was 2 minutes per tunnel. Given the described application protocols in the study report it's difficult to understand how applications could have been made to each of the tunnels in such a short timeframe.

On -2 and -1 DATs the mean daily temperature was 13.9-14.1 °C (minimum daily temperatures were 11.4-21.1 °C); additionally, cloud cover on these days was 100%. OECD Guidance Document No. 75 notes that daytime temperatures below 15 °C may inhibit honeybee foraging activity. Additionally, there was substantial rainfall on 3 (20 mm) and 7 DATs (8 mm) during the exposure phase of the study. While these adverse environmental conditions would have theoretically affected all treatment groups equally, nevertheless they result in some uncertainty regarding the degree of foraging activity of colonies at the time of applications, and during the exposure phase of the study.

Study results indicate that the primary reference item (fenoxycarb) resulted in the following significant ($p < 0.05$) adverse effects relative to control colonies: reduced numbers of adult worker bees/colony/d on 19, 26, 69, and 93 DATS; reduced numbers of pupae/colony/d on 8, 14, 19, and 26 DATs; and, reduced numbers of brood cells per colony on 4, 8, and 26 DATs. As previously discussed, fenoxycarb treatments also appeared to adversely affect brood development, but this effect relative to the negative control could not be statistically tested. Furthermore, data from the single dimethoate-treated tunnel appeared to also show adverse treatment effects on honeybee colonies, notably increased adult worker bee mortality during the exposure phase of the study; however, as this treatment was not replicated, it could not be included in statistical analyses. Collectively, these responses due to reference item treatments suggest that honeybee colonies in this study were exposed to test materials, and that to some degree the test system was able to detect treatment effects associated with both of the reference toxicants; however, the degree of adverse effects was somewhat minimal, and the reviewer believes that there is some uncertainty as to how effectively honeybee colonies in this study were exposed to afidopyropen and reference item (fenoxycarb and dimethoate) treatments applied as part of the study.

Reviewer's Conclusions:
The semi-field (tunnel) bee brood study was initiated in June 2014 with the formulated end-use product BAS 440 00 I (VERSYS™, 9.8% afidopyropen) applied both during active bee foraging (*i.e.*, daytime) and in

the absence of active foraging (*i.e.*, evening). Bee colonies in the negative control, reference item (fenoxycarb: 300 g a.i./ha nominal & dimethoate: 480 g a.i./ha nominal), and 10 g a.i./ha BAS 440 00 I treatments were assessed at multiple time points; treatment rates were not confirmed analytically. The exposure phase was seven days (0 – 7 DAT), and the post-exposure monitoring phase was 27 days for all endpoints except for colony strength and condition, which was monitored for a total of 93 days after applications.

There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen (daytime or evening applications) treatment groups and the negative control during the pre-application or exposure phases of the study; during the monitoring phase, mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, lower by 15%) in daytime application afidopyropen tunnels compared to control tunnels. There was reportedly no mortality of pupae measured in afidopyropen tunnels at any point in the study. There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen (daytime or evening applications) tunnels and the negative control during the pre-application phase of the study, but during the exposure phase of the study, mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 27% lower) in daytime application afidopyropen tunnels relative to negative control tunnels. With the exception of one instance (19 DAT), there were no significant ($p<0.50$) differences in colony strength (mean number of adult worker bees or pupae/colony/d) or condition (mean number of brood or food cells/colony/d) in test item (daytime or evening applications) tunnels relative to the negative control.

The mean brood index and brood compensation index was significantly ($p<0.05$) different (*i.e.*, lower by 35-38 and 29-44%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies, and the mean brood termination rate was significantly ($p<0.05$) different (*i.e.*, higher by 130-169%, respectively) in colonies that received a daytime application of afidopyropen relative to control colonies. Overall effects on brood development from evening applications of afidopyropen were similar to effects from daytime applications, though of slightly lower magnitude (*i.e.*, lower brood index and brood compensation index, and higher brood termination rate), but these effects were not significantly different from those in negative control colonies due to higher variance around treatment means. Finally, afidopyropen treatments resulted in sublethal behavioral effects after application on the day of treatment (0aa DAT) in the daytime application tunnels. Within 30 minutes of applications 10-30 bees in each tunnel were motionless, showed reduced ability to respond to stimulation, fell off of crop plants, exhibited impaired locomotion and cramping; these sublethal effects were observed to have occurred only through the end of the day of applications (*i.e.*, 0 DAT).

There were adverse weather conditions during the pre-application period (*i.e.*, daily temperatures < 14 °C and 100% cloud cover), and 3-7 DAT (20 and 8 mm of rainfall, respectively). There was also substantial rainfall (> 5 mm) periodically throughout the monitoring phase of the study. Additionally, because nominal treatment levels of afidopyropen and dimethoate were not verified analytically, there is some uncertainty regarding actual exposure levels. However, measured test item residue levels do indicate that colonies were exposed to the afidopyropen treatments.

The study was generally consistent with OECD Guidance Document 75, and indicates that honey bee colonies treated with formulated afidopyropen at 10 g a.i./ha during active bee flight (*i.e.*, in the daytime) exhibited significant adverse effects on foraging activity, and brood development. Overall effects on brood development from evening applications of afidopyropen were similar to effects from

daytime applications, though of slightly lower magnitude (*i.e.*, lower brood index and brood compensation index, and higher brood termination rate), but these effects were not significantly different from those in negative control colonies. Based on this study and the noted statistically significant effects, the NOAEL is <10 g a.i./ha for applications during active bee flight.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

APPENDIX I. Output of Statistics Verified by the Reviewer

Adult Honeybee Mortality (no. dead bees/colony/d)

```
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
   Min     1Q Median     3Q    Max
-54.54  -4.59   1.22   5.67 417.15

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   33.2196     7.2952   4.554 6.49e-06 ***
trtmntref a   -0.4392     3.5239  -0.125  0.90086
trtmntref b   49.5068     5.1585   9.597  < 2e-16 ***
trtmnttest a  -1.2500     3.2625  -0.383  0.70176
trtmnttest b   1.3243     3.2625   0.406  0.68496
time-2        -1.5000     9.9225  -0.151  0.87990
time-3        -3.6875     9.9225  -0.372  0.71031
time-4       -25.7500     9.9225  -2.595  0.00971 **
time -1ba    -25.5000     9.9225  -2.570  0.01043 *
time 0aa1    -28.1875     9.9225  -2.841  0.00467 **
time 0aa2    -22.8750     9.9225  -2.305  0.02152 *
time 0aa3     -2.5000     9.9225  -0.252  0.80117
time 0aa4    -10.6875     9.9225  -1.077  0.28191
time 0ba     -26.6250     9.9225  -2.683  0.00751 **
time1         26.1250     9.9225   2.633  0.00870 **
time10       -26.2500     9.9225  -2.645  0.00839 **
time11       -19.3125     9.9225  -1.946  0.05212 .
time12       -22.3750     9.9225  -2.255  0.02453 *
time13       -25.8750     9.9225  -2.608  0.00936 **
time14       -18.5000     9.9225  -1.864  0.06279 .
time15       -22.7500     9.9225  -2.293  0.02224 *
time16       -16.7500     9.9225  -1.688  0.09196 .
time17       -11.6875     9.9225  -1.178  0.23935
time18       -21.1250     9.9225  -2.129  0.03370 *
time19       -21.9375     9.9225  -2.211  0.02745 *
time2         -3.9375     9.9225  -0.397  0.69165
time20       -25.6250     9.9225  -2.583  0.01007 *
time21       -24.8125     9.9225  -2.501  0.01269 *
time22       -29.1250     9.9225  -2.935  0.00347 **
time23       -25.6875     9.9225  -2.589  0.00989 **
time24       -28.3750     9.9225  -2.860  0.00440 **
time25       -27.3750     9.9225  -2.759  0.00599 **
time26       -25.8750     9.9225  -2.608  0.00936 **
time27       -27.1250     9.9225  -2.734  0.00646 **
time3          4.3125     9.9225   0.435  0.66401
time4          6.6250     9.9225   0.668  0.50462
time5          5.6250     9.9225   0.567  0.57102
time6         -8.8125     9.9225  -0.888  0.37486
time7         -6.3750     9.9225  -0.642  0.52083
time8        -16.6250     9.9225  -1.675  0.09441 .
time9        -18.9375     9.9225  -1.909  0.05684 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 28.07 on 551 degrees of freedom
Multiple R-squared:  0.2972,     Adjusted R-squared:  0.2462
F-statistic: 5.825 on 40 and 551 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.41968, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 906.29, df = 4, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 1319, df = 36, p-value < 2.2e-16
```

Pre-application Phase
Kruskal-Wallis rank sum test ~ test item I
Kruskal-Wallis chi-squared = 0.71577, df = 2, p-value = 0.6992

Kruskal-Wallis rank sum test ~ test item II
Kruskal-Wallis chi-squared = 0.0089794, df = 1, p-value = 0.9245

Exposure Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.19245, df = 2, p-value = 0.9083

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.081765, df = 1, p-value = 0.7749

Monitoring Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 30.267, df = 3, p-value = **1.213e-06**

Pairwise comparisons using Wilcoxon rank sum test

	cont	ref a	test a
ref a	**0.016**	-	-
test a	**0.043**	0.436	-
test b	**0.021**	**6.2e-05**	**5.0e-05**

P value adjustment method: holm

Foraging Activity (bees/m²/d)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:

Min	1Q	Median	3Q	Max
-8.0360	-1.4127	0.0833	1.6540	8.4616

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	9.6495	0.3976	24.269	< 2e-16	***
trtmntref a	0.6521	0.2412	2.703	0.006987	**
trtmnttest a	-2.4802	0.2233	-11.104	< 2e-16	***
trtmnttest b	0.4722	0.2271	2.079	0.037877	*
time-2	3.7111	0.5285	7.021	4.08e-12	***
time-3	1.3111	0.5285	2.481	0.013281	*
time-4	6.0444	0.5285	11.436	< 2e-16	***
time0aa1	3.6667	0.5285	6.937	7.22e-12	***
time0aa10	3.6005	0.8258	4.360	1.44e-05	***
time0aa11	4.0172	0.8258	4.865	1.33e-06	***
time0aa12	3.6005	0.8258	4.360	1.44e-05	***
time0aa13	2.9339	0.8258	3.553	0.000399	***
time0aa14	1.2672	0.8258	1.535	0.125210	
time0aa2	2.0444	0.5285	3.868	0.000117	***
time0aa3	1.2000	0.5285	2.270	0.023398	*
time0aa4	0.9556	0.5285	1.808	0.070924	.
time0aa5	2.8444	0.5285	5.382	9.22e-08	***
time0aa6	1.8667	0.5285	3.532	0.000432	***
time0aa7	-3.1111	0.5285	-5.886	5.41e-09	***
time0aa8	3.6005	0.8258	4.360	1.44e-05	***
time0aa9	3.2672	0.8258	3.957	8.15e-05	***
time0ba	4.7109	0.5768	8.167	9.58e-16	***
time1aa1	4.1111	0.5285	7.778	1.85e-14	***
time1aa2	7.5556	0.5285	14.295	< 2e-16	***
time1aa3	5.9778	0.5285	11.310	< 2e-16	***
time2	5.2000	0.5285	9.838	< 2e-16	***
time3	2.2222	0.5285	4.204	2.86e-05	***
time4	-0.1111	0.5285	-0.210	0.833537	
time5	6.8222	0.5285	12.908	< 2e-16	***
time6	6.2667	0.5285	11.857	< 2e-16	***

```
time7          -3.1778     0.5285  -6.012 2.57e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.507 on 986 degrees of freedom
Multiple R-squared:  0.6099,     Adjusted R-squared:  0.5981
F-statistic: 51.39 on 30 and 986 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.99298, p-value = 9.709e-05

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 123.5, df = 3, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 518.34, df = 27, p-value < 2.2e-16

Pre-application Phase
Kruskal-Wallis rank sum test ~ test item I
Kruskal-Wallis chi-squared = 0.63766, df = 2, p-value = 0.727

Kruskal-Wallis rank sum test ~ test item II
Kruskal-Wallis chi-squared = 0.55334, df = 1, p-value = 0.457

Exposure Phase
Kruskal-Wallis rank sum test ~ test item I
Kruskal-Wallis chi-squared = 65.809, df = 2, p-value = 5.127e-15

Pairwise comparisons using Wilcoxon rank sum test

       cont    ref a
ref a  5.7e-05 -
test a 2.4e-09 5.7e-12

P value adjustment method: holm

Kruskal-Wallis rank sum test ~ test item II
Kruskal-Wallis chi-squared = 8.1911, df = 1, p-value = 0.00421

Pairwise comparisons using Wilcoxon rank sum test

       cont
test b 0.0042

P value adjustment method: holm

-------------------------------------------------------------------------------------------
Colony Strength (no. adult bees/colony/d)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-4314.0  -866.4    95.4   931.5  4338.6

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   10502.6      510.8  20.561  < 2e-16 ***
trtmntref a   -3293.0      460.8  -7.146 1.07e-10 ***
trtmnttest a   -414.9      426.7  -0.973 0.332947
trtmnttest b   -133.6      426.7  -0.313 0.754794
time4           630.1      623.2   1.011 0.314233
time8          1589.9      623.2   2.551 0.012118 *
time14         2077.5      623.2   3.334 0.001172 **
time19          585.1      623.2   0.939 0.349890
time26         1027.6      623.2   1.649 0.102036
time69         3292.4      623.2   5.283 6.56e-07 ***
time93         2347.5      623.2   3.767 0.000268 ***
---
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1707 on 109 degrees of freedom
Multiple R-squared:  0.4967,     Adjusted R-squared:  0.4505
F-statistic: 10.76 on 10 and 109 DF,  p-value: 1.619e-12

Shapiro-Wilk normality test
W = 0.98537, p-value = 0.2219

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 2.5949, df = 3, p-value = 0.4584

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 64.928, df = 7, p-value = 1.555e-11

aov ~ -2 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 2517902  839301    1.08  0.398
Residuals   11 8548229  777112

aov ~ 4 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 3301093 1100364   4.134 0.0344 *
Residuals   11 2928235  266203
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0     899.9      394.1   2.284    0.105
test a - cont == 0   -478.0      364.8  -1.310    0.453
test b - cont == 0    140.8      364.8   0.386    0.963
(Adjusted p values reported -- single-step method)

aov ~ 8 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        3  1289574  429858    0.42  0.742
Residuals   11 11257589 1023417

aov ~ 14 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        3 12763233 4254411   3.354 0.0591 .
Residuals   11 13954955 1268632

aov ~ 19 DAT
            Df   Sum Sq  Mean Sq F value  Pr(>F)
group        3 31782097 10594032   9.542 0.00214 **
Residuals   11 12212311  1110210
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -3947.3      804.8  -4.905  0.00105 **
test a - cont == 0  -1125.0      745.1  -1.510  0.34773
test b - cont == 0   -281.3      745.1  -0.377  0.96494
 (Adjusted p values reported -- single-step method)

aov ~ 26 DAT
            Df   Sum Sq  Mean Sq F value  Pr(>F)
group        3 63663544 21221181   11.46 0.00104 **
Residuals   11 20364919  1851356
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
```

```
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -5624.9     1039.2  -5.413   <0.001 ***
test a - cont == 0   -506.2      962.1  -0.526    0.915
test b - cont == 0  -1462.2      962.1  -1.520    0.343
 (Adjusted p values reported -- single-step method)

aov ~ 69 DAT
            Df    Sum Sq  Mean Sq F value   Pr(>F)
group        3 155087518 51695839   30.09 1.34e-05 ***
Residuals   11  18897797  1717982
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0  -7659.17    1001.08  -7.651   <0.001 ***
test a - cont == 0    84.25     926.82   0.091    0.999
test b - cont == 0   900.00     926.82   0.971    0.663
 (Adjusted p values reported -- single-step method)

aov ~ 93 DAT
            Df    Sum Sq  Mean Sq F value   Pr(>F)
group        3 123421857 41140619   18.79 0.000123 ***
Residuals   11  24089434  2189949
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -7556.3     1130.3  -6.686   <0.001 ***
test a - cont == 0   -787.5     1046.4  -0.753    0.799
test b - cont == 0   -478.0     1046.4  -0.457    0.941
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)

--------------------------------------------------------------------------------
Colony Strength (no. juveniles/colony/d)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-2077.50  -659.85   -37.15   556.96  2655.00

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    6287.7      285.8  21.997  < 2e-16 ***
trtmntref a   -1750.2      257.9  -6.787 6.23e-10 ***
trtmnttest a   -573.0      238.8  -2.400   0.0181 *
trtmnttest b   -233.8      238.8  -0.979   0.3296
time4          -442.5      348.7  -1.269   0.2072
time8         -1728.7      348.7  -4.957 2.64e-06 ***
time14        -2925.0      348.7  -8.388 1.96e-13 ***
time19        -2718.7      348.7  -7.796 4.06e-12 ***
time26         -660.0      348.7  -1.893   0.0611 .
time69        -3735.0      348.7 -10.711  < 2e-16 ***
time93        -3540.0      348.7 -10.151  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 955 on 109 degrees of freedom
Multiple R-squared:  0.7331,     Adjusted R-squared:  0.7086
F-statistic: 29.94 on 10 and 109 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.98593, p-value = 0.2486
```

```
Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 1.5225, df = 3, p-value = 0.6771

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 26.783, df = 7, p-value = 0.0003646

aov ~ -2 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3  238148   79383   0.114   0.95
Residuals   11 7681289  698299

aov ~ 4 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        3   349840  116613   0.097   0.96
Residuals   11 13261113 1205556

aov ~ 8 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 8123994 2707998   3.958 0.0387 *
Residuals   11 7525459  684133
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -1818.7      631.7  -2.879   0.0384 *
test a - cont == 0   -520.3      584.9  -0.890   0.7150
test b - cont == 0    196.9      584.9   0.337   0.9745
 (Adjusted p values reported -- single-step method)

aov ~ 14 DAT
            Df   Sum Sq Mean Sq F value  Pr(>F)
group        3 25221586 8407195   8.689 0.00306 **
Residuals   11 10642852  967532
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -3665.6      751.3  -4.879  0.00133 **
test a - cont == 0   -900.0      695.5  -1.294  0.46207
test b - cont == 0   -731.2      695.5  -1.051  0.61096
 (Adjusted p values reported -- single-step method)

aov ~ 19 DAT
            Df   Sum Sq Mean Sq F value   Pr(>F)
group        3 27011127 9003709   43.31 2.21e-06 ***
Residuals   11  2286826  207893
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -3965.6      348.2 -11.388   <0.001 ***
test a - cont == 0  -1842.2      322.4  -5.714   <0.001 ***
test b - cont == 0  -1743.7      322.4  -5.409   <0.001 ***
 (Adjusted p values reported -- single-step method)

aov ~ 26 DAT
            Df   Sum Sq Mean Sq F value  Pr(>F)
group        3 22373086 7457695   7.605 0.00499 **
Residuals   11 10786289  980572
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                     Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -3346.9      756.3  -4.425  0.00295 **
test a - cont == 0   -843.7      700.2  -1.205  0.51466
test b - cont == 0   -337.5      700.2  -0.482  0.93219
 (Adjusted p values reported -- single-step method)

aov ~ 69 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 1463906  487969   1.784  0.208
Residuals   11 3007969  273452

aov ~ 93 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 1023258  341086   0.641  0.605
Residuals   11 5856680  532425
```

Colony Condition - Brood (no. cells/colony/d as brood)

```
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-1273.71  -359.78    -0.82   353.32  1938.05

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   1896.68     117.85  16.094  < 2e-16 ***
trtmntref a   -594.73     106.32  -5.594 6.31e-08 ***
trtmnttest a  -275.10      98.43  -2.795  0.00563 **
trtmnttest b  -274.22      98.43  -2.786  0.00579 **
time4         -427.50     143.77  -2.973  0.00326 **
time8         -511.88     143.77  -3.560  0.00045 ***
time14         326.25     143.77   2.269  0.02419 *
time19         352.50     143.77   2.452  0.01496 *
time26        -157.50     143.77  -1.095  0.27446
time69        -315.00     143.77  -2.191  0.02946 *
time93       -1095.00     143.77  -7.616 6.83e-13 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 556.8 on 229 degrees of freedom
Multiple R-squared:  0.4459,     Adjusted R-squared:  0.4217
F-statistic: 18.43 on 10 and 229 DF,  p-value: < 2.2e-16
Shapiro-Wilk normality test
W = 0.9895, p-value = 0.07897

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 5.2115, df = 3, p-value = 0.1569

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 15.467, df = 7, p-value = 0.03046

aov ~ -2 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3  490957  163652   0.455  0.716
Residuals   26 9349277  359588

aov ~ 4 DAT
            Df  Sum Sq Mean Sq F value  Pr(>F)
group        3 4990887 1663629   4.734 0.00915 **
Residuals   26 9137285  351434
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
```

```
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -1204.7      320.2  -3.763  0.00247 **
test a - cont == 0   -506.2      296.4  -1.708  0.23450
test b - cont == 0   -562.5      296.4  -1.898  0.16741
 (Adjusted p values reported -- single-step method)

aov ~ 8 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 2700501  900167   3.996 0.0182 *
Residuals   26 5857339  225282
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0    -876.6      256.3  -3.420   0.0058 **
test a - cont == 0   -400.8      237.3  -1.689   0.2423
test b - cont == 0   -281.3      237.3  -1.185   0.5128
 (Adjusted p values reported -- single-step method)

aov ~ 14 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3 1148449  382816   1.169   0.34
Residuals   26 8510801  327338

aov ~ 19 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        3  1101199  367066   0.789  0.511
Residuals   26 12098848  465340

aov ~ 26 DAT
            Df  Sum Sq Mean Sq F value  Pr(>F)
group        3 5200031 1733344   6.037 0.00292 **
Residuals   26 7465078  287118
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                   Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -1228.1      289.4  -4.244   <0.001 ***
test a - cont == 0   -562.5      267.9  -2.100    0.114
test b - cont == 0   -478.1      267.9  -1.785    0.205
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)

aov ~ 69 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3  725730  241910   0.752  0.531
Residuals   26 8365254  321741

aov ~ 93 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        3  316301  105434   0.838  0.485
Residuals   26 3270059  125771
```

Colony Condition – Food (no. cells/colony/d as food)

```
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-9217.7 -2393.4  -419.3  2148.1 12350.6

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
```

```
(Intercept)    1719.4      848.5   2.026  0.04389 *
trtmntref a    -364.5      765.5  -0.476  0.63447
trtmnttest a    199.5      708.7   0.282  0.77858
trtmnttest b    530.9      708.7   0.749  0.45462
time4           776.3     1035.2   0.750  0.45411
time8          1220.6     1035.2   1.179  0.23957
time14         1751.3     1035.2   1.692  0.09206 .
time19         1980.0     1035.2   1.913  0.05703 .
time26         2077.5     1035.2   2.007  0.04594 *
time69         3393.8     1035.2   3.278  0.00121 **
time93         7080.0     1035.2   6.839 7.17e-11 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4009 on 229 degrees of freedom
Multiple R-squared:  0.2172,     Adjusted R-squared:  0.1831
F-statistic: 6.355 on 10 and 229 DF,  p-value: 1.308e-08

Shapiro-Wilk normality test
W = 0.97501, p-value = 0.0003065

Bartlett test of homogeneity of variances
Bartlett's K-squared = 9.7328, df = 3, p-value = 0.02098

Bartlett test of homogeneity of variances
Bartlett's K-squared = 160.49, df = 7, p-value < 2.2e-16

aov ~ -2 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.74765, df = 3, p-value = 0.8619

aov ~ 4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.8432, df = 3, p-value = 0.6056

aov ~ 8 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.8442, df = 3, p-value = 0.6054

aov ~ 14 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 2.1829, df = 3, p-value = 0.5353

aov ~ 19 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.5944, df = 3, p-value = 0.6607

aov ~ 26 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.49243, df = 3, p-value = 0.9206

aov ~ 69 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.8563, df = 3, p-value = 0.6028

aov ~ 93 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.2054, df = 3, p-value = 0.361

--------------------------------------------------------------------------------
Brood Index (bi)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-2.0370 -0.4072  0.0585  0.3265  2.0530

Coefficients:
```

```
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.9742     0.2659  11.184 1.46e-15 ***
trtmntref a   -3.2510     0.3028 -10.737 6.59e-15 ***
trtmnttest a  -1.2012     0.2803  -4.285 7.73e-05 ***
trtmnttest b  -1.0012     0.2803  -3.572 0.000764 ***
time8          0.2353     0.2895   0.813 0.419932
time14         0.2113     0.2895   0.730 0.468625
time19         0.6840     0.2895   2.363 0.021850 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7929 on 53 degrees of freedom
Multiple R-squared:  0.7001,     Adjusted R-squared:  0.6662
F-statistic: 20.63 on 6 and 53 DF,  p-value: 2.724e-12

Shapiro-Wilk normality test
W = 0.96061, p-value = 0.05038

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 116.99, df = 3, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 1.746, df = 3, p-value = 0.6268

4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.0912, df = 3, p-value = 0.0281

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.30 -     -
test a 0.30 0.30  -
test b 0.69 0.30  1.00

8 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 8.8658, df = 3, p-value = 0.03113

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.25 -     -
test a 0.17 0.25  -
test b 1.00 0.25  1.00

P value adjustment method: holm_

14 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 8.8817, df = 3, p-value = 0.03091

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.24 -     -
test a 0.18 0.24  -
test b 1.00 0.24  1.00

P value adjustment method: holm_

19 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.0548, df = 3, p-value = 0.02857

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.24 -     -
test a 0.18 0.24  -
test b 1.00 0.24  1.00

P value adjustment method: holm
```

```
--------------------------------------------------------------------------------
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-2.13708 -0.50990  0.00542  0.56813  1.95292

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.9033     0.3105   9.351 7.99e-12 ***
trtmnttest a  -1.2012     0.3105  -3.869 0.000374 ***
trtmnttest b  -1.0012     0.3105  -3.225 0.002441 **
time8          0.2942     0.3585   0.821 0.416534
time14         0.2650     0.3585   0.739 0.463900
time19         0.8550     0.3585   2.385 0.021672 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.8781 on 42 degrees of freedom
Multiple R-squared:  0.3561,     Adjusted R-squared:  0.2794
F-statistic: 4.645 on 5 and 42 DF,  p-value: 0.00183

Shapiro-Wilk normality test
W = 0.97297, p-value = 0.3291

Bartlett test of homogeneity of variances
Bartlett's K-squared = 24.04, df = 2, p-value = 6.023e-06

Bartlett test of homogeneity of variances
Bartlett's K-squared = 1.5006, df = 3, p-value = 0.6821

4 DAT
Welch Two Sample t-test ~ test item I
t = 2.8585, df = 4.6426, p-value = 0.03864

Welch Two Sample t-test~ test item II
t = 1.5335, df = 4.5206, p-value = 0.1918

8 DAT
Welch Two Sample t-test ~ test item I
t = 3.5905, df = 4.2485, p-value = 0.02072

Welch Two Sample t-test ~ test item II
t = 1.1815, df = 3.9321, p-value = 0.3039

14 DAT
Welch Two Sample t-test ~ test item I
t = 3.7885, df = 3.9493, p-value = 0.01975

Welch Two Sample t-test ~ test item II
t = 1.2021, df = 3.9703, p-value = 0.2961

19 DAT
Welch Two Sample t-test ~ test item I
t = 3.5357, df = 3.856, p-value = 0.02561

Welch Two Sample t-test ~ test item II
t = 1.1787, df = 4.1037, p-value = 0.3023

--------------------------------------------------------------------------------
Brood Compensation Index (bci)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-1.88617 -0.30520  0.05198  0.36738  1.76383
```

```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    3.0107     0.2443  12.325  < 2e-16 ***
trtmntref a   -3.2844     0.2781 -11.810  < 2e-16 ***
trtmnttest a  -1.2250     0.2575  -4.758 1.55e-05 ***
trtmnttest b  -0.9919     0.2575  -3.852 0.000318 ***
time8          0.2367     0.2659   0.890 0.377503
time14         0.3807     0.2659   1.431 0.158165
time19         1.0073     0.2659   3.788 0.000390 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7283 on 53 degrees of freedom
Multiple R-squared:  0.7494,     Adjusted R-squared:  0.7211
F-statistic: 26.42 on 6 and 53 DF,  p-value: 2.658e-14

Shapiro-Wilk normality test
W = 0.95168, p-value = 0.0187

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 32.94, df = 3, p-value = 3.316e-07

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 1.1444, df = 3, p-value = 0.7664

4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.075, df = 3, p-value = 0.02831

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.34 -     -
test a 0.34 0.34  -
test b 0.69 0.34  1.00

P value adjustment method: holm_

8 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 8.8658, df = 3, p-value = 0.03113

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.25 -     -
test a 0.17 0.25  -
test b 1.00 0.25  1.00

P value adjustment method: holm_

14 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.85, df = 3, p-value = 0.01989

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.29 -     -
test a 0.17 0.29  -
test b 0.69 0.29  0.69

P value adjustment method: holm_

19 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.025, df = 3, p-value = 0.02896

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.29 -     -
test a 0.17 0.29  -
```

```
test b 1.00 0.29  1.00

P value adjustment method: holm
-------------------------------------------------------------------------------------
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-1.97458 -0.36292  0.05812  0.42979  1.67542

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.9290     0.2830  10.351 3.96e-13 ***
trtmnttest a  -1.2250     0.2830  -4.329 9.08e-05 ***
trtmnttest b  -0.9919     0.2830  -3.505 0.001098 **
time8          0.3058     0.3267   0.936 0.354597
time14         0.4683     0.3267   1.433 0.159143
time19         1.1775     0.3267   3.604 0.000824 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.8003 on 42 degrees of freedom
Multiple R-squared:  0.4555,     Adjusted R-squared:  0.3907
F-statistic: 7.028 on 5 and 42 DF,  p-value: 7.508e-05

Shapiro-Wilk normality test
W = 0.96216, p-value = 0.1234

Bartlett test of homogeneity of variances
Bartlett's K-squared = 14.964, df = 2, p-value = 0.000563

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.50819, df = 3, p-value = 0.9171

4 DAT
Welch Two Sample t-test ~ test item I
t = 3.032, df = 4.9165, p-value = 0.02965

Welch Two Sample t-test ~ test item II
t = 1.5835, df = 4.4149, p-value = 0.1818

8 DAT
Welch Two Sample t-test ~ test item I
t = 3.8751, df = 4.5551, p-value = 0.01399

Welch Two Sample t-test ~ test item II
t = 1.2123, df = 3.8244, p-value = 0.2949

14 DAT
Welch Two Sample t-test ~ test item I
t = 8.2429, df = 5.9074, p-value = 0.0001867

Welch Two Sample t-test ~ test item II
t = 1.6801, df = 3.221, p-value = 0.1852

19 DAT
Welch Two Sample t-test ~ test item I
t = 3.4776, df = 3.6667, p-value = 0.02922

Welch Two Sample t-test ~ test item II
t = 1.1064, df = 3.9782, p-value = 0.3309

-------------------------------------------------------------------------------------
Brood Termination Rate (btr, %)

Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
```

```
   Min     1Q Median     3Q    Max
-39.69 -10.04  -0.49  11.53  44.82

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    19.204      6.397   3.002  0.00408 **
trtmntref a    78.886      7.283  10.832 4.78e-15 ***
trtmnttest a   29.258      6.743   4.339 6.46e-05 ***
trtmnttest b   23.301      6.743   3.456  0.00109 **
time8           1.959      6.964   0.281  0.77953
time14          2.509      6.964   0.360  0.72003
time19          2.509      6.964   0.360  0.72003
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 19.07 on 53 degrees of freedom
Multiple R-squared:  0.6947,     Adjusted R-squared:  0.6601
F-statistic:  20.1 on 6 and 53 DF,  p-value: 4.32e-12

Shapiro-Wilk normality test
W = 0.93676, p-value = 0.003889

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 123.75, df = 3, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ time
Bartlett's K-squared = 0.038545, df = 3, p-value = 0.998

4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.1914, df = 3, p-value = 0.02685

Pairwise comparisons using Wilcoxon rank sum test
       cont ref a test a
ref a  0.25 -     -
test a 0.17 0.25  -
test b 0.97 0.25  1.00

P value adjustment method: holm_

8 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.0385, df = 3, p-value = 0.02878

Pairwise comparisons using Wilcoxon rank sum test
   cont ref a test a
ref a  0.25 -     -
test a 0.17 0.25  -
test b 1.00 0.25  1.00

P value adjustment method: holm_

14 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 8.8817, df = 3, p-value = 0.03091

Pairwise comparisons using Wilcoxon rank sum test
cont ref a test a
ref a  0.24 -     -
test a 0.18 0.24  -
test b 1.00 0.24  1.00

P value adjustment method: holm_

19 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 8.8817, df = 3, p-value = 0.03091

Pairwise comparisons using Wilcoxon rank sum test
```

```
       cont ref a test a
ref a  0.24 -     -
test a 0.18 0.24  -
test b 1.00 0.24  1.00

P value adjustment method: holm

-------------------------------------------------------------------------------
Call: lm(formula = value ~ trtmnt + time, data = z)

Residuals:
   Min     1Q Median     3Q    Max
-39.86 -12.73   0.90  12.29  45.25

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    18.782      7.571   2.481  0.01721 *
trtmnttest a   29.258      7.571   3.864  0.00038 ***
trtmnttest b   23.301      7.571   3.078  0.00367 **
time8           2.449      8.743   0.280  0.78075
time14          3.109      8.743   0.356  0.72390
time19          3.109      8.743   0.356  0.72390
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 21.42 on 42 degrees of freedom
Multiple R-squared:  0.2864,     Adjusted R-squared:  0.2014
F-statistic: 3.371 on 5 and 42 DF,  p-value: 0.01194

Shapiro-Wilk normality test
W = 0.94713, p-value = 0.03077

Bartlett test of homogeneity of variances
Bartlett's K-squared = 33.385, df = 2, p-value = 5.629e-08

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.027565, df = 3, p-value = 0.9988

4 DAT
Welch Two Sample t-test ~ test item I
t = -3.451, df = 4.5949, p-value = 0.02086

Welch Two Sample t-test ~ test item II
t = -1.2435, df = 4.0026, p-value = 0.2816

8 DAT
Welch Two Sample t-test ~ test item I
t = -3.3512, df = 4.102, p-value = 0.02746

Welch Two Sample t-test ~ test item II
t = -1.1696, df = 4.0701, p-value = 0.306

14 DAT
Welch Two Sample t-test ~ test item I
t = -3.489, df = 3.8209, p-value = 0.02708

Welch Two Sample t-test ~ test item II
t = -1.1537, df = 4.1077, p-value = 0.3113

19 DAT
Welch Two Sample t-test ~ test item I
t = -3.489, df = 3.8209, p-value = 0.02708

Welch Two Sample t-test ~ test item II
t = -1.1537, df = 4.1077, p-value = 0.3113
```